Examination of αRL, αRS

18h br. culture of αRL + αRS, non aerated.

Microscopic examination (stained smear + wet mount): Cells of αRS are perhaps slightly smaller, though the difference is not very evident.

Plated:

				[Titer]
αRL	$:10^5$	.05 cc	1 – 84	1.7×10^8
αRS	$:10^5$	.05 cc	2 – 110	2.2×10^8

Purpose: find out the reason ~~for~~ of the different size of the colonies of αRL + αRS.

The number of αRB under various conditions.

I.

24h culture of [B 1cal.] = B_{1c}

0	.4cc B_{1c} + .1cc α = B_α	
5	B_{1c} :10^6 .05cc	41 - 266 42 - 266
10	[α: ~~25~~ x 10^3] .1cc # B_{1c} .05cc	43 - 15 + 26 44 - 17 + 13
20	α .1cc # B_α .05cc	45 - 8 + 14 46 - 5 + 10
23	B_α :10^6 B	49 - 81

Results - $[B_{1c}] = 5.3 \times 10^9$

$[\alpha R]_{43\text{-}44} = 390\ L + 390\ S$ $\quad \alpha RL/B = 6 \times 10^{-8}$; $\alpha RS/B = 7 \times 10^{-8}$

$[\alpha R]_{45\text{-}46} = 160\ L + 300\ S$ $\quad \alpha RL/B = 3 \times 10^{-8}$; $\alpha RS/B = 6 \times 10^{-8}$

$[\alpha_{inf.\ cent.}]_{B_\alpha} = 1.6 \times 10^9$

II.

24h culture of $B_{stock} = B_{st}$

B_{st} :10^6 .05cc	47 - 175	$[B_{st}] = 3.5 \times 10^9$
[α:50] .1cc # [B_{st} :20] .05cc	48 - 1 + 20	$[\alpha R] = 400\ L + (8 \times 10^3)\ S$

$\alpha RL/B = 1.2 \times 10^{-7}$

$\alpha RS/B = 2.5 \times 10^{-6}$

A Comparison between the Numbers of αRB from Various Samples of the Same Culture and from Different Cultures.

-120 .05cc 24h aer. B → 10 cc broth, aer. = B_I

0 $[B_I : 10^4]$ .1cc → .9cc broth, tubes

	Pl.#	[B]	# L+S.	# L+S
(1)	1 -180	3.6 × 10^8	11 - 500 (1)	
(2)	2 -206	4.1 "	12 - 0+2	
(3)	3 -212	4.2 "	13 - 0+14*	
(4)	4 -224	4.5 "	14 - 2+0	
(5)	[5 - 302]	[6.0 "]	15 - 0+0	
(6)	6 - 195	3.9 "	16 - 1+1	31 - 1+0
(7)	7 - 300	6.0 "	17 - 1+0	32 - 0+0
(8)	8 - 196	3.9 "	18 - 0+0	33 - 0+0
(9)	9 - 242	4.8 "	19 - 0+0	34 - cont.
(10)	10 - 178	3.6 "	20 - 0+5	35 - cont.
				36 - 0+0
(11)	30 - 138			37 - 0+0
				38 - 0+0
				39 - 0+0
				40 - 2+0

$[B_I : 10^4]$ .2cc → 1.8 cc broth, tube

5 min. $B_I : 10^4$ .1cc plate 61-38

Tubes (1)÷(11) incubated 15h at 37°. Then plated as follows:

Tubes (1)÷(10) : (n) : 10^5 .05cc plated alone Plates 1÷10
(n) pure .1cc " # α : 50 .1cc " 11÷20

Tube (11) : (11) : 10^5 .05cc plated alone Plate 30
(11) pure, ten samples of .1cc pl. # α " 31÷40

results shown above.

(1) - 500 colonies, of small size, but apparently of type L. Some of them very transparent (partially lysed?). 2 colonies taken to 2 slants.

The result, though in agreement with the expectation, doesn't prove definitely that the number of resistants ~~[illegible]~~ in various samples of one culture follows a Poisson distribution, while the number of resistants in different cultures show wild fluctuations.

* The 14 colonies appear after 48h. 2 of them, tested after growth on slants for sensitivity to α and γ, are: one sensitive to α and to γ.
" " resist " " " also partly to γ.

Microscopic observation - Number of divisions of B on agar[α]-seeded plates -

Plate 1 : .05 cc 24[h] non aerated B - Inc. at 37°
" 2 : .1cc (α:50) + same - " " -

Observations :

	Plate 1	Plate 2
1[h]	Microcolonies of 2-16 cells -	Groups of 1-4-8 cells -
2[h]	Microcolonies of 8-32 cells (confluent)	Few microcolonies - Single cells - Most have apparently lysed -

(10)

Fluctuations in the production of α Resistant B

-130' .03 cc 24 hr aer. B → 10 cc br., aer. at 37 = B_I

0 [B_I : 2.5×10^3] .1 cc → 10 cc broth, aer. - tubes (1) - (10) -

14 hr. 5 Plate as follows.

		[B]	[L]	[S]	[L]/[B]	[S]/[B]
(1) : 10^6 " #α:20	1-155 11 - 26 + 3 = 29 12 - 20 + 1	3.1×10^9	4.4×10^2	5×10^1	1.4×10^{-7}	1.6×10^{-8}
(2) : 10^6 " #α:20	2 - 175 13 - 11 + 30 14 - 10 + 24 = 41	3.5×10^9	2×10^2	5.3×10^2	$.6 \times 10^{-7}$	15×10^{-8}
(3) : 10^6 " #α:20	3 - 260 15 - 17 + 6 16 - 11 + 6 = 17	5.2×10^9	2.8×10^2	1.2×10^2	$.5 \times 10^{-7}$	2×10^{-8}
(4) : 10^6 " #α:20	4 - 171 17 - 5 + 15 18 - 5 + 20 = 20	3.4×10^9	1.0×10^2	3.5×10^2	$.3 \times 10^{-7}$	10×10^{-8}
(5) : 10^6 " #α:20	5 - 160 19 - 12 + 19 20 - 14 + 24 = 31	3.2×10^9	2.6×10^2	4.2×10^2	$.8 \times 10^{-7}$	13×10^{-8}
(6) : 10^6 " #α:20	6 - 66 21 - 4 + 1 22 - 0 + 0	1.3×10^9				
(7) : 10^6 " #α:20	7 - 185 23 - 5 + 25 = 30	3.7×10^9	1.0×10^2	5.0×10^2	$.3 \times 10^{-7}$	13×10^{-8}
(8) : 10^6 " #α:20	8 - 4 25 - 3 + 0	8×10^7	6×10^1	—	8×10^{-7}	—
(9) : 10^6 " #α:20	9 - 247 27 - 3 + 4 = 7	4.9×10^9	6×10^1	8×10^1	$.1 \times 10^{-7}$	1.6×10^{-8}

over

10a (continued)

		[B]	[L]	[S]	$\frac{[L]}{[L]+[S]} L_{31}$	[S]/[B]
(10) : 10^6	10 - 272	5.4×10^9				
" #α:20	31 - 10+4		200	80	1	1
	32 - 11+4		220	80	1.1	1
	33 - 8+5		160	100	.8	1.25
	34 - 17+4		340	80	1.7	1
	35 - 9+6		180	120	.9	1.5
	36 - 10+4		200	80	1	1
	37 - 17+9		340	180	1.7	2.25
	38 - 12+4		240	80	1.2	1.5
	39 - 10+10		200	200	1.0	2.5
	40 - 9+4		180	80	.9	1
Average tube (10)			226	108	4.2×10^{-8}	2×10^{-8}

$[L]+[S] = 3.32 \times 10^2$

α :20 alone 41 - 0

Tube (10), 10 samples:

	L	S	L + S
m_{av}	11.3	5.4	16.7
$(m_{av})^2$	127.65	29.16	280
$(m^2)_{av}$	136.9	33.8	295
variance $= (m - m_{av})^2_{av} =$ $= (m^2)_{av} - (m_{av})^2$	9.25	4.64	15

$\chi^2 = 8.2$, $P = .52$ $\chi^2 = 8.6$, $P = .47$ $\chi^2_{10} = 9$, $P = .4$

Tubes ① ② ③ ④ ⑤ ⑦ ⑨

m_{av}	8.35	13.6	2
$(m_{av})^2$	69.7	185	
$(m^2)_{av}$	103	247	
variance	33.3	62	
$\propto N$	2.8	4.1	
$\propto [aN]^2$ = variance calc.	54.5	117	

	[L]	[S] (aver., tubes 1, 2, 3, 4, 5, 7, 9, 10)	[L+S]
$\bar{m}$ =	10.4	13.4	23.8
$(\bar{m})^2$ =	108	179	568
$\overline{(m^2)}$ =	141	264	676
var. =	33	85	108
var.$-\bar{m}$ =	23	72	84
aN	3.4	3.9	6.2
$C(aN)^2$	92	120	308

$\chi^2_8 = 33.4$

$\chi^2_8 = 43$

$P = .01$

$\chi^2_8 = 19$

$a_s = \frac{6.2}{2\times10^8} = 3.1 \times 10^{-8}$

$aN_t = 570$

$a_t = \frac{570}{4\times10^{10}} = 1.42 \times 10^{-8}$

$C(a_t N_s)^2 = 640$

Fermentation reactions of B, αRL, αRS

	Dextrose	Sucrose	Lactose	Mannitol	Glycerol
B	AG	AG	AG	AG	AG
αRL	AG	AG	AG	AG	AG
αRS	AG	AG	AG	AG	AG

Fluctuation of α Resistants -

-150 .25 cc 24^4 aer. B → 10 cc broth aer. at 37° = B_I

0 [B_I : 10^4], 1 cc → 10 cc broth, aer. = ① – ⑩

9h Plate as follows.

	:10^6	# α : 10	[B]	[L]	[S]	[L+S] : [B]
①	1 - 272	11 - 23 + 7	5.4×10^9	4.6×10^2	1.4×10^2	1.1×10^{-7}
②	2 - 144	12 - 3 + 7	2.9×10^9	6×10^1	1.4×10^2	$.7 \times 10^{-7}$
③	3 - 216	13 - 34 + 6	4.3×10^9	6.8×10^2	1.2×10^2	1.8×10^{-7}
④	4 - 200	(14 - 9 + 36 +?)	4.0×10^9	1.8×10^2	7.2×10^1	2.2×10^{-7}
⑤	5 - 141	15 - 172 + 11	9.8×10^9	3.4×10^3	2.2×10^2	13×10^{-7}
⑥	6 - 160	16 - 8 + 4	3.2×10^9	1.6×10^2	8×10^1	1.3×10^{-7}
⑦	7 - 225	17 - 124 + 49	4.5×10^9	2.5×10^3	1×10^3	7.8×10^{-7}
⑧	8 - 220	18 - 21 + 2	2.4×10^9	4.2×10^2	4×10^1	1.0×10^{-7}
⑨	9 - 218	19 - 36 + 21	3.4×10^9	7.2×10^2	4.2×10^2	2.6×10^{-7}
⑩	10 - 194	20 - 42 + 4	3.9×10^9	9.0×10^2	1.3×10^2	2.6×10^{-7}
		22 - 49 + 7				
		23 - 44 + 9				
		24 - 44 + 4				
		25 - 59 + 6				
		26 - 37 + 7				
		27 - 43 + 6				
		28 - 43 + 8				
		29 - 48 + 8				
		30 - 41 + 6				

(11a)

+ one of the 36 small colonies, plated again, showed to be really a large col. strain -

L + S

$m_{av} = 51.4$

$(m_{av})^2 = 2650$

$(m^2)_{av} = 2677$

Variance = 27

$\chi^2_{.10} = 5.3$

$p = .8$

	Large R.	Sm. R.
m_{av}	45	6.4
m_{av}^2	2025	40.96
$(m^2)_{av}$	2057	43.0
variance	12	2.04
	$\chi^2 = 2.7$, $P = .77$	$\chi^2 = 3.2$, $P = .96$

10 samples from tube (10)

m_{av}	47.5	14
m_{av}^2	2256	196
$(m^2)_{av}$	6056	445
variance	3800.	249
	$\chi^2 = 790$	$\chi^2 = 178$
$aN = 10$		3.8
$C(aN)^2 = 1000$		145

tubes (1) - (10)+ average for (10).

$$N = \frac{3.98 \times 10^9}{20} = 2 \times 10^8$$

$$a_L = \frac{10}{2 \times 10^8} = 5 \times 10^{-8}$$

$$a_S = \frac{3.8}{2 \times 10^9} = 1.9 \times 10^{-8}$$

$$12400 = \bar{m}_t = (aN)_t \ln 10 (aN)_t$$

$$a_t N_t = 1650$$

$$a_t = \frac{1650}{4 \times 10^{10}} = 4.125 \times 10^{-8}$$

$$C (a_t N_s)^2 = 680$$

(conclusion)

L + S

m_{av} 62

$(m_{av})^2 = 3920$

$(m^2)_{av} = 7481$

variance = 3561 ↳ variance − m_{av} = 3500

$aN = 13$ $\qquad \chi^2_{10} = 584$

$C(a^2N^2) = 1690$

$a = \frac{13}{2 \times 10^8} = 6.5 \times 10^{-8}$ (calculated from the samples)

~~$a = \frac{184}{4 \times 10^9} = 3.35 \times 10^{-8}$ (calculated from the per cc)~~

$a_t = \frac{1300}{4 \times 10^{10}} = 3.25 \times 10^{-8}$ (calculated per 10 cc) $\qquad aN_t = 1390$

~~The total number of mutants in the 10 tubes is 9500 (wrong)~~

Total number of mutants $= 1.25 \times 10^5$

aN (total bacteria) $= 11000$

$a_{total} = \frac{11000}{4 \times 10^{11}} = 2.75 \times 10^{-8}$ $\qquad \frac{a}{a_{tot}} = 2.7$

~~$C(a_t N_t)^2 = 420$~~

(12) 3/4

Test for number of division of B on α-seeded agar

PST

-5 Plate (1) : (α:15) .05 cc
" (2) : (α:150) .05 cc
" (3) (α:1500) .05 cc

0 Pl. (1)(2)(3) seeded with .05 cc B (~5x10⁸/cc) -
" (4) also (control -

Observations :

0 Single small bacteria in (1), (2), (3), (4)

45 min Single + double large bact. " (2), (3), (4)
Most bacteria disappeared in (1)

90 min Microcol. with many long forms in (4)
Fewer and smaller microcol. in (3)
Lysis almost complete in (2)
" " in (1) (few remaining cells) -

180 min Same story -

Test for reverse-mutations (resistant ⇒ α-sensitive) by growth-test of α

-150	.025cc (α RL : 50) → 10 cc brs., aer., = L_I		.025cc (α RS : 50) → 10 cc brs., aer., = S_I	
-5	(α : 10) : 2.5×10^5	1 - 197 2 - 236 [α] = 1.1×10^9		
0	.1cc (α : 10) → L_I		.1cc (α : 10) → S_I	
5	L_I : 2.5×10^3	3 - 224	S_I : 2.5×10^3	4 - 162
60	"	5 - 188 (cloudy, ~10^{10})	"	6 - 198 (cloudy, ~10^7)
120	"	7 - 204	"	8 - 239
195	"	9 - 207	"	10 - 165

[L] initial ≃ 10^6

[L] final ~ 5×10^8

[α] input = 1.1×10^7

[α] final$_{L_I}$ (aver.) = 1.05×10^7

[S] initial ≃ 10^6

[S] final ~ 5×10^8

[α] input = 1.1×10^7

[α] final$_{S_I}$ = 1.0×10^7

No growth of α in any of the tubes —

No detectable production of α sensitive —

Test for increase of α-resistant B with standing

PST hrs						
0	24^h B : 10^7, .1cc → 10cc br., 2 tubes (1) + (2), aer. at 37°C.					
12	(1) : 10^6	1 - 50 2 - 61		(1) # α	11 - 3+4 12 - 4+2	
	(2) : 10^6	3 - 90 4 - 100	1.1×10^9	(2) # α	13 - 10+24 14 - 10+26	70
~~20~~ 4	Keep (2) at 37°C, no aeration.					
30	(2) : 10^6	5 - 73 6 - 109	1.8×10^9	(2) # α	15 - 6+20 16 - 8+16	50
54	(2) : 10^6	7 - 37 8 - 52	9×10^8	(2) # α	17 - 8+7 18 - 6+6	27
104	(2) : 2.5×10^5	9 - 20	1×10^8	(2) # α	19 - 1+0	1

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Number of resistants after plating with various amounts of α

α: 50	# B24 h	1 - 30 + 27
α: 500	# " "	2 - 18 + 32
α: 5000	# " "	3 - 25 + 46
α: 5×10^4	# " "	4 - disc. growth

Fluctuations of α-Resistant in 10 cultures

-150 : 0.25 cc 24h aer. B → 10 cc bx., aer at 37° = B_I

0 B_I : 10^4 , .1 cc → 10 cc broth, ten tubes = ① → ⑩ -

8.5h Plate as follows -

	:10^6	# α : 50	[B]	[L]	[S]	[L+S]/[B]
①	1-98	11-3+3	2×10^9	60	60	6×10^{-8}
②	2-187	12-4+1	3.8×10^9	80	20	2.6×10^{-8}
③	3-251	13-6+4	5×10^9	120	80	4×10^{-8}
④	4-98	14-2+6	2×10^9	40	120	8×10^{-8}
⑤	5-191	15-10+14	3.3×10^9	200	280	12×10^{-8}
⑥	6-133	16-8+(5) Med.	2.7×10^9	160 ?	100 ?	9.5×10^{-8}
⑦	7-156	17-1+164	3.1×10^9	20	3300	105×10^{-8}
⑧	8-144	18-5+10 (Med)	2.9×10^9	100 ?	200 ?	70×10^{-8}
⑨	9-132	19-5+1	2.6×10^9	100	20	4.5×10^{-8}
⑩	10-73	20-9+1	1.5×10^9	180	20	13×10^{-8}
Average			2.9×10^9			

Calculations : next page

L + S

m_{av} 26.2

m_{av}^2 685 ~

$(m^2)_{av}$ 2880

variance 2200 $\chi^2_{10} = 840$

$aN = 6.3$

$C(aN)^2 = 400$

$$a = \frac{6.3}{N_{sample}} = \frac{6.3}{2.45 \times 10^9} = 2.4 \times 10^{-8}$$

Total number of mutants : 5.25×10^4
" " " bacteria : 2.9×10^{11}

$aN_{total} = 4800$

$$a = \frac{4800}{2.9 \times 10^{11}} = 2.4 \times 10^{-8} \qquad \frac{a}{a_{tot}} = 3.1$$

N per tube $= 2.9 \times 10^{10}$
$\bar{m}$ per tube $= 5.24 \times 10^3$

$5.23 \times 10^3 = x \log 10\, x$; $x = 600 = aN_t$

$$a_t = \frac{600}{2.9 \times 10^{10}} = 2.1 \times 10^{-8}$$

$$C(a_t N_t)^2 = 92$$

α-Res B from various types of cultures

I. B slant with about 100-200 colonies - suspend in broth -

Plate : B : 6.25×10^6 1-167 $[B] = 2.1 \times 10^{10}$
α # B 2-16+29 $[R] = 900$

II. B_{24h} in broth .05cc # α 3-5+17

III. " " in M8 (same initial inoculum) .05cc # K 4-39+0

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Fluctuations in α-Res-B from M8 cultures

-2.5h .025 cc 24h aer. B in M8 → 10 cc M8 = B_I

0 B_I [~10^7]: 2×10^4 M8 – 20 tubes with .2 cc each – Incubate at 37° in moist chamber.

14h Plate as follows.

Tube	$.5 \times 10^5$	.08 cc #α	[B]	[L]	[S]	[L+S]
1	1-221	11- 1+0	2.2×10^9	12	–	12
2	2-236	12- 0+0	2.4×10^9	–	–	–
3	3-336	13- 3+0	3.4×10^9	38	–	38
4	4-202	14- 0+0	2.0×10^9	–	–	–
5	5-335	15- 0+0	3.35×10^9	–	–	–
6	6-323	16- 5+0	3.2×10^9	63	–	63
7	7-237	17- 0+0	2.4×10^9	–	–	–
8	8-?	18- 5+0 (nibbled)		63	–	63
9	9-271	19- 0+0	2.7×10^9	–	–	–
10	10-352	20- 6+0	3.5×10^9	75	–	75
11		21- 107+0		1350	–	1350
12		22- 0+0		–	–	–
13		23- 0+0		–	–	–
14		24- 0+0		–	–	–
15		25- 1+0		12	–	12
16		26- 0+0		–	–	–
17		27- 0+0		–	–	–
18		28- 1+63		12	790	802
19		29- 0+0		–	–	–
20		30- 0+35		–	440	440

average 2.8×10^9

$m_{av} = 11.35$

$(m_{av})^2 = 128$

$(m^2)_{av} = 834$

variance $= 706$

$\chi^2_{20} = 1240$

$aN = 2.8$

$C(aN)^2 = 157$

$N = \frac{2.8 \times 10^9}{\text{[illegible]}} \times .08 = 2.24 \times 10^8$

$a_t = 1.25 \times 10^{-8}$

Average number of mutants per tube = 28

Total number of bacteria per tube = 5.6×10^8

$aN_{\text{per tube}} = 6$

$a = \frac{6}{5.6 \times 10^8} = 1.08 \times 10^{-8}$

$C(a_t N_s)^2 = 116$

Fluctuations of α-Resistant B in M8

−120 mm.	.05 cc 24h aer. B in M8 ⟶ 10 cc M8, at 37 = B_I
0	B : 4×10^4 M8 – .2 cc in each of 18 tubes (1) – (18)
16 hrs	12 tubes show definite growth – 1 shows faint turbidity – 5 tubes are clear – Plate from the 12 grown tubes –

Tube	$:10^6$ (.05cc)	.08cc # α	[B]	[L]	[S]	[L+S]	[L+S]/[B]
(1)	21-126	1- 1+0	2.5×10^9	12	–	12	4.5×10^{-9}
(2)	22-111	2- 0+0	2.2×10^9	–	–	–	–
(3)	23-147	3-0+0	2.9×10^9	–	–	–	–
(4)	24-118	4-7+0	2.4×10^9	84	–	84	3.5×10^{-8}
(5)		5-0+0		–	–	–	
(6)		6-303+0		3636	–	3600	
(7)		7-0+0		–	–	–	
(8)		8-0+0		–	–	–	
(9)		9-0+3		–	36	36	
(10)		10-48+0		580	–	580	
(11)		11-1+0		12	–	12	
(12)		12-4+0		48	–	48	
Average			2.5×10^9	360	3		

$m_{av.} = 30$

$(m_{av})^2 = 900$

$(m^2)_{av} = 7750$

Variance $= 6850$

$\chi^2_{12} = 2650$

$aN = 6.8$

$C(aN)^2 = 550$

$N_{per\ sample} = 2.5 \times 10^9 \times .08 = 2 \times 10^8$

$a_{sample} = 3.4 \times 10^{-8}$

$N_{per\ tube} = 5 \times 10^8$

$aN_{per\ tube} = 15$

$a_{per\ tube} = \frac{15}{5.0 \times 10^8} = 3.0 \times 10^{-8}$

$C(a_t N_s)^2 = 330$

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Plating of B with various dilutions of α

24 h culture of B from (B colony stock).

		Av. total
.075 cc [α: 50] + .05 cc B	1 – 18 + 36 2 – 28 + 32	~~59.5~~
.075 cc [α: 200] + .05 cc B	3 – 27 + 59 4 – 15 + 41	61
.075 cc [α: 4000] + .05 cc B	5 – 19 + 57 6 – 37 + 54	83.5
.075 [α: 2×10^4] + .05 cc B	7 – 39 + 58 8 – 57 + 41	97.5

Test for α-resistant mutants after UV irradiation of B.

Irradiation of a 5h culture of B (from ~10^5 cells).
" 1.5 minutes and 3 minutes.

Plating:

Control : 10^5	.05cc	1 – 158 2 – 150
1.5 min : 50	"	3 : 0 4 – 0
3 min prime	"	5 – 0 6 – 1
Control .05cc + α : 50		7 – 17+2 8 – 24+3

Cultures:

.1cc 1.5 min in 10 cc broth = $B_{1.5}$

.1cc 3 min " " " = B_3 (no growth)

.025cc control : 2.5×10^3 " " = $B_{control}$

After aeration for 17 hrs, plate as follows:

α + $B_{1.5}$	.05 cc	9 – 18+7 10 – 19+6
α + B_{cont}	.05 cc	13 – 85+8 14 – 75+10

α-Resistant colonies from B plated with various amounts of α

3.5h culture of B (small inoculum) plated with various amounts of α-

B : 2.5×10^5	1 - 290	$[B] = 1.45 \times 10^9$
B pure + α pure	51 - 7 + 8 52 - 6 + 10	$[\alpha R] = 3.2 \times 10^2$
B pure # α : 100	53 - 9 + 1 54 - 8 + 10	$[\alpha R] = 3.8 \times 10^2$
B pure # α : 4000	55 - 6 + 23 56 - 16 + 25	$([\alpha R] = 6.5 \times 10^2)$

Microscopic observation after 100 min shows that lysis is already well advanced in plates 51-52, also in plates 53-54. Plates 55-56 show an almost continuous layer of bacteria, with some holes.

Notice the difference of the number of mutants on plates 55 and 56.

The amount of α Resistant from a growing culture, at various times.

Two cultures of B started from a 24^4 culture (small inoc.) - Labeled I and II.

150 min. — Plate: I: 2.5×10^4 11-204 $[I] = 1.0 \times 10^8$
II: 5×10^4 12-181 $[II] = 1.8 \times 10^8$

I # α 1-0+1
II # α 2-0+1

210 min I: 2.5×10^5 13-290 $[I] = 1.5 \times 10^9$
II: 2.5×10^5 14-226 $[II] = 1.2 \times 10^9$

I # α 8-2+4
II # α 4-3+7

300 min I: 10^6 15-124 $[I] = 2.5 \times 10^9$
II: 10^6 16-195 $[II] = 4 \times 10^9$

I # α 5-14+19
II # α 6-18+18

Apparently the expected increased in the proportion of mutants is masked by the fluctuations.

(21)

Fluctuation of α R B from broth cultures, aerated and not-aerated.

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0 young culture B (~2×10^7) : 4×10^4, then { .2 cc added to 5 tubes with 10 cc bs. (I÷V); .2 cc put in small tubes (1÷19)

Incubate I÷V with aeration, 1÷19 in incubator.

13^h Plate as follows:

	for B count	+ α
1		1-0+0
2		2-0+0
3	(:10^5) 31-274	3-0+0
4		4-0+0
5		5-7+1
6		6-1+0
7		7-0+0
8		8-1+0
9		9-0+0
10		10-0+15
11		11-0+0
12		12-0+0
13		13-2+17
14		14-0+0
15		15-0+0
16		16-17+0
17		17-0+11
18		18-0+0
19		19-0+0

(21a)

[B] = 5.5×10^8

I	(:10^6) 32-162	51-10+28 ✓
II		52-14*+14
III		53-13+12 ✓
IV		54-83°+16 ✓ (99+8)
V		55-10+3

(21b)

[B] = 3.2×10^9

* 12 of these 14 are "medium".
° 79 " " 83 " "

5/7 (cont.)

Analysis -

I - 19 small cultures -

		αRL	αRS	αRL + αRS
	$\bar{m}$	1.47	2.3	3.77
	$\bar{m}^2$	2.2	5.35	11.2
	$\overline{(m^2)}$	18.1	33.5	55.8
	var.	15.9	28.15	44.6
	var-$\bar{m}$	14.43	25.85	40.83
	χ^2	187	217	234
per sample	aN	.6	.83	1.2
per sample	$c(aN)^2$	6.85	13	27.4
per sample	N	2.75×10^7	2.75×10^7	2.75×10^7
per sample	a	2.2×10^{-8}	3.0×10^{-8}	4.4×10^{-8}
per tube (total)	$\bar{m}$	5.9	9.2	15.1
per tube (total)	N	1.1×10^8	1.1×10^8	1.1×10^8
per tube (total)	aN	1.7	2.4	3.6
per tube (total)	a	1.55×10^{-8}	2.2×10^{-8}	3.3×10^{-8}
	$c(a_{\pm} N_3)^2$			15.5

(cont.)

5/7 (concl.)

Analysis (cont.) -

II. 5 large cultures

	αRL	αRS	αRL + αRS
$\bar{m}$	29.2	13	42.2
$\bar{m}^2$	853	169	1781
$\overline{m^2}$	2073	239	2894
var	1220	70	1113
var $-\bar{m}$	1190	57	1071
χ^2	204	22	127
per sample: aN	8	4.25	10.7
per sample: $(aN)^2$	320	90	560
per sample: N	1.6×10^8	1.6×10^8	1.6×10^8
per sample: a	5×10^{-8}	2.65×10^{-8}	6.7×10^{-8}
per tube (total): $\bar{m}$	5850	2600	8440
per tube (total): N	3.2×10^{10}	3.2×10^{10}	3.2×10^{10}
per tube (total): aN	700	350	975
per tube (total): a	2.2×10^{-8}	1.1×10^{-8}	3.0×10^{-8}
$C(a_t N_s)^2$	62	15	114

~~32~~ 20
20
18
48
25
81
1024
100
64
289
1298
64
3610
640
64
3540
635
638

1.6×10^3
6.4×10^4
6.4×10^5

Sample
r ~~[illegible]~~ $= 2.15$
$r^2 = 4.6$

$\overline{r^2}$ ~~variance~~ $= 35.7$
variance $= 31.1$

culture
$r = 8.6$

$8.60 = x \log 100x$

$x = aN =$ ~~[illegible]~~ 1.9
$N = 2.8 \times 10^8$

$a_t N_s = .48$

$C a_t^2 N_s^2 = 23$

5/13

Fluctuations of α Res. B from 100 M8 cultures

0 8h culture of B in M8 (~ 5 × 10^6) : 10^4, distributed in 108 tubes, in portions of .2 cc. Incubate at 37°.

20 hrs Plating as follows:

I. Tube (7) : 10^6 207 - 81
" (31) : 10^6 231 - 78
" (61) : 10^6 261 - 68
" (87) : 10^6 287 - 56

} Average: 1.4×10^9 B/cc
2.8×10^8 B/tube
7×10^7 B/sample

II. Plate # α : 10 .05 cc :

1 ✓	0+0	21 ✓	0+0	41 ✓	0+1	61 ✓	0+4•	81 ✓	1+0
2 ✓	0+0	22 ✓	1*+0	42 ✓	0+0	62 ✓	1+18	82 ✓	0+5
3 ✓	0+0	23 ✓	1+0	43 ✓	0+0	63 ✓	0+0	83 ✓	0+0
4 ✓	0+0	24 ✓	0+0	44 ✓	0+0	64 ✓	0+0	84 ✓	0+0
5+6 ✓	0+0	25 ✓	1+0	45	17*	65 ✓	0+0	85 ✓	3+0
6 ✓	0+1	26 ✓	0+0	46 ✓	0+0	66 ✓	0+0	86 ✓	0+0
7 ✓	0+0	27 ✓	0+0	47 ✓	36•+0	67 ✓	1+0	87 ✓	2+0
8 ✓	1+0	28 ✓	0+0	48+49 ✓	6+0	68 ✓	1+0	88 ✓	0+0
9 ✓	9+0	29 ✓	0+0	49 ✓	0+0	69 ✓	0+0	89 ✓	0+0
10 ✓	0+0	30 ✓	1•+0	50 ✓	1+0	70 ✓	0+0	90 ✓	0+0
11 ✓	2+0	31 ✓	0•+0	51 ✓	0+0	71 ✓	4+0	91 ✓	0+0
12 ✓	1+797	32 ✓	0+0	52 ✓	5+3	72 ✓	4•+0	92 ✓	0+0
13 ✓	0+0	33 ✓	0+0	53 ✓	0+0	73 ✓	0+0	93 ✓	1+0
14 ✓	2+0	34 ✓	0+0	54 ✓	2+0	74 ✓	0+0	94 ✓	1•+0
15 ✓	1+0	35 ✓	1*+0	55 ✓	0+0	75 ✓	8+0	95 ✓	0+1
16 ✓	0+0	36 ✓	0+0	56 ✓	0+0	76 ✓	0+8	96 ✓	1•+0
17 ✓	0+0	37 ✓	0+0	57 ✓	3+0	77 ✓	0+0	97 ✓	0+0
18 ✓	0+0	38 ✓	8*+0	58 ✓	0+0	78 ✓	0+0	98 ✓	0+0
19 ✓	12+19+1	39 ✓	2+0	59 ✓	1+0	79 ✓	0+0	99 ✓	0+0
20 ✓	1+0	40 ✓	1+0	60 ✓	1+0	80 ✓	0+0	100 ✓	0+0
								101 ✓	0+0
								102 ✓	0+0
								103 ✓	2+0
								104 ✓	1+0

* transparent, milder drops • medium
• nibbled